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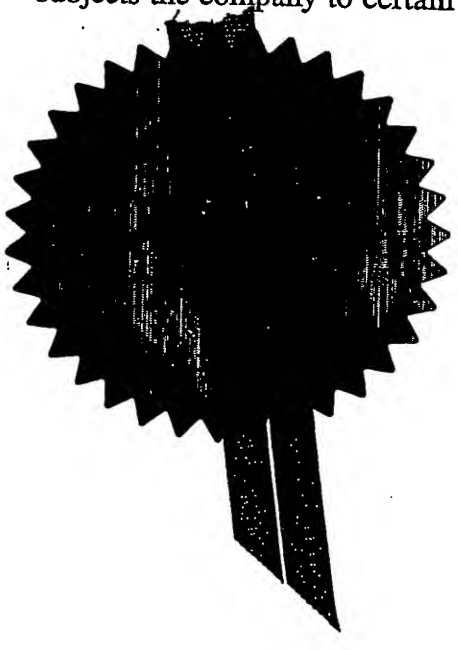

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1. Your reference — 24 MAR 2003 — SMC 60575/GB/P1

2. Patent application number *(The Patent Office will fill in this part)* — 0306657.8

24MAR03 E794526 1 B02744
P01/7700 0.00-0306657.8

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Avecia Limited
Hexagon House
Blackley
Manchester, M9 8ZS

Patents ADP number *(if you know it)* — 07764137001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — PROCESS AND COMPOUNDS

5. Name of your agent *(if you have one)* — REVELL, Christopher

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Avecia Limited
Hexagon House
PO Box 42
Blackley
Manchester M9 8ZS

Patents ADP number *(if you know it)* — 6969885001 — 7764137001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or

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c) any named applicant is a corporate body.
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
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Request for substantive examination *(Patents Form 10/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature G Terry Date 24/3/03

Avecia Limited Authorised Signatory

12. Name and daytime telephone number of person to contact in the United Kingdom: K.M.Pinder/G.Terry 0161 721 1361/2

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APPLICANTS

AVECIA LIMITED

TITLE

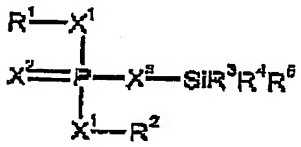
PROCESS AND COMPOUNDS

PROCESS AND COMPOUNDS

The present invention concerns a method for the synthesis of oligonucleotides, oligonucleotide derivatives, and methods for the preparation thereof.

According to one aspect of the present invention there is provided an oligonucleotide comprising at least one internucleotide phosphorus atom protected with a group of formula $-X^aSiR^3R^4R^5$ wherein X^a represent O or S, and R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more. Preferably at least 50%, more preferably at least 75% and most preferably 100% of the internucleotide phosphorus atoms are protected with a group of formula $-SiR^3R^4R^5$.

A particular embodiment of the present invention provides compounds of Formula (1):



Formula (1)

wherein:

R^1 and R^2 independently are nucleoside or oligonucleotide moieties;

R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more;

X^a represents O or S, preferably O;

each X^1 independently is O, S or NR^n, where R^n represents H or C_{1-4} alkyl, preferably each X^1 being O; and

X^2 is O or S, preferably S.

Nucleoside or oligonucleotide moieties that can be represented by R^1 and R^2 include deoxyribonucleosides, oligodeoxyribonucleotides, ribonucleosides, oligoribonucleoside, and oligonucleotides comprising mixtures of deoxyribo- and ribonucleosides. The nucleosides or oligonucleotides may be modified by one or modifications known in the field of oligonucleotide chemistry, for example ribonucleosides or oligoribonucleotides may be modified at one or more of the 2'-positions by the presence of 2'-alkoxy group, such as a methoxy or methoxyethoxy group. Deoxyribonucleosides or oligodeoxyribonucleotides may be modified at the 2'-position by the presence of a substituent, such as a halo group, especially a fluoro group, or by an alkenyl group such as an allyl group. Abasic nucleoside moieties may also be present. In many

embodiments, the nucleosides or oligonucleotides represented by R^1 and R^2 will represent the natural D-isomer. However, either or both of R^1 and R^2 may represent an unnatural isomer, for example an L-isomer or a B-anomer, either in whole or in part. One or both of R^1 and R^2 may comprise one or more protecting groups. Examples of such protecting groups, and the positions which they can be employed to protect, are well known to those skilled in the art, and include trityl, monomethoxytrityl and dimethoxytrityl groups, levulinoyl groups, isobutyryl groups, benzoyl groups, acetyl groups and carbonate groups, such as BOC and especially FMOC.

In many embodiments, X^1 connects the 3'-position of a ribose or deoxyribose moiety of R^1 to the phosphorus, P. However, it will be recognised that X^1 may connect the 5'-position of a ribose or deoxyribose moiety of R^1 to the phosphorus, P.

In many embodiments, X^1 connects the 5'-position of a ribose or deoxyribose moiety of R^2 to the phosphorus, P. However, it will be recognised that X^1 may connect the 3'-position of a ribose or deoxyribose moiety of R^2 to the phosphorus, P.

Either of R^1 and R^2 may be attached to a solid support, commonly via a cleavable linker. In many embodiments, R^2 is attached to a solid support via a cleavable linker, preferably via the 3'-position of a ribose or deoxyribose moiety. Examples of cleavable linkers include base labile linkers such as succinyl linkers, and acid labile linkers such as trityl linkers.

Hydrocarbyl groups which can be represented by one or more of R^3, R^4 and R^5 include any optionally substituted hydrocarbyl groups that allow the P(III) centre to react with a sulphurising agent or oxidation agent, especially optionally substituted alkyl groups, optionally substituted aryl groups and mixtures thereof, such as aralkyl, especially benzyl, groups.

When at least one of R^3, R^4 and R^5 represents an optionally substituted alkyl group, it is preferably an optionally substituted C_{1-12} alkyl, more preferably an optionally substituted C_{1-6}alkyl and particularly an optionally substituted C_{1-4}alkyl group.

When at least one of R^3, R^4 and R^5 represents an optionally substituted aryl group, it is preferably an optionally substituted phenyl group.

R^3, R^4 and R^5 may be the same or different.

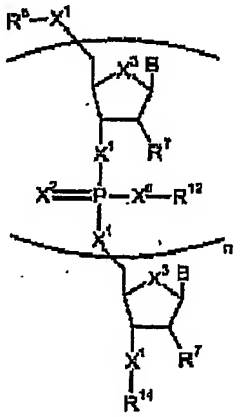
Preferably the total number of carbon atoms in R^3, R^4 and R^5 is 5 or greater.

In a preferred embodiment one of R^3, R^4 and R^5 is *tert*-butyl and the other two are methyl.

Optional substituents for R^3, R^4 and R^5 are preferably selected from the group consisting of alkyl (preferably C_{1-4}-alkyl), optionally substituted alkoxy (preferably C_{1-4}-alkoxy), optionally substituted aryl (preferably phenyl), optionally substituted aryloxy (preferably phenoxy), polyalkylene oxide (preferably polyethylene oxide or polypropylene oxide), carboxy, phosphato, sulpho, nitro, cyano, halo, ureido, $-SO_2F$, hydroxy, ester, $-NR^aR^b$, $-COR^a$, $-CONR^aR^b$, $-NHCOR^a$, carboxyester, sulphone, and $-SO_2NR^aR^b$ wherein

R^a and R^b are each independently H or optionally substituted alkyl (especially C_{1-4}-alkyl) or, in the case of $-CONR^aR^b$ and $-SO_2NR^aR^b$, R^a and R^b together with the nitrogen atom to which they are attached represent an aliphatic or aromatic ring system; or a combination thereof.

Preferred compounds of Formula (1) include compounds of formula (2):



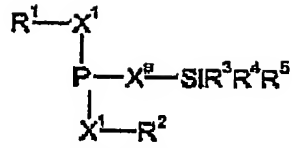
Formula (2)

In compounds of formula (2), X^a for each occurrence is independently -O- or –S-. Preferably X^a is O at each occurrence. X^1 for each occurrence is, independently, -O-, -S- or NR^n, where R^n represents H or C_{1-4} alkyl. Preferably, X^1 is -O- at every occurrence. X^2 for each occurrence is O or S, preferably S. X^3 for each occurrence is, independently, -O-, -S-, $-CH_2-$, or $-(CH_2)_2-$. Preferably, X^3 is -O- at every occurrence. In a more preferred embodiment, X^1 and X^3 are all -O- at every occurrence. R^6 is H, an alcohol protecting group, an amino protecting group or a thio protecting group. Preferably, R^6 is a protecting group which is removable under conditions orthogonal to the group of formula X^a-$SiR^3R^4R^5$. R^7 for each occurrence is, independently, -H, -F $-OR^8$, $-NR^9R^{10}$, $-SR^{11}$, or a substituted or unsubstituted aliphatic group, such as methyl or allyl. R^{12} for each occurrence is, independently, a phosphorus protecting group, such as a group of formula $-CH_2CH_2CN$, a substituted or unsubstituted aliphatic group, $-R^{13}$, $-CH_2CH_2-Si(CH_3)_2C_6H_5$, $-CH_2CH_2-S(O)_2-CH_2CH_3$ or $-CH_2CH_2-C_6H_4-NO_2$, provided that at least one R^{12} represents a group of formula $-SiR^3R^4R^5$, in which R^3, R^4 and R^5 are as previously defined. In certain embodiments, each R^{12} represents a group of formula $-SiR^3R^4R^5$. In certain other embodiments, only one R^{12} represents a group of formula $-SiR^3R^4R^5$, advantageously being located at the 5'-terminal internucleotide phosphorus. R^8 for each occurrence is, independently, -H, a substituted or unsubstituted aliphatic group (e.g., methyl, ethyl, methoxyethyl or allyl), a substituted or unsubstituted aryl group, a substituted or

unsubstituted aralkyl, an alcohol protecting group, or $-(CH_2)_q-NR^xR^y$. R^9 and R^{10} for each occurrence are each, independently, -H, a substituted or unsubstituted aliphatic group, or an amine protecting group. Alternatively, R^9 and R^{10} taken together with the nitrogen to which they are attached are a heterocyclyl group. R^{11} for each occurrence is, independently, -H, a substituted or unsubstituted aliphatic group, or a thio protecting group. R^{13} is for each occurrence is, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group or a substituted or unsubstituted aralkyl group. R^x and R^y are each, independently, -H, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aralkyl group, a substituted or unsubstituted heteroaralkyl group or an amine protecting group. Alternatively, R^x and R^y taken together with the nitrogen to which they are attached form a heterocyclyl group. q is an integer from 1 to about 6. B is -H, a natural or unnatural nucleobase, or a protected natural or unnatural nucleobase. R^{14} is H a hydroxy protecting group, a thio protecting group, an amino protecting group, $-(CH_2)_q-NR^xR^y$, a solid support, or a cleavable linker attached to a solid support, such as a group of the formula $-Y-L-Y-R^{15}$. Y for each occurrence is, independently, a single bond, -C(O)-, $-C(O)NR^{16}-$, -C(O)O-, $-NR^{16}-$ or -O-. L is a linker which is preferably a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group, for example a trityl group. More preferably, L is an ethylene group. R^{16} is -H, a substituted or unsubstituted aliphatic group or a substituted or unsubstituted aromatic group. R^{16} is any solid support suitable for solid phase oligonucleotide synthesis known to those skilled in the art. Examples of suitable solid supports include controlled-pore glass, polystyrene, microporous polyamide, such as poly(dimethylacrylamide), and polystyrene coated with polyethylene. In many embodiments, R^{14} represents a cleavable linker, such as a succinyl, oxaloyl or trityl linker, attached to a solid support. n is a positive integer, preferably from 1 to 100, for example up to 75, commonly up to 50, and particularly from 8 to 40.

Natural and unnatural nucleobases that can be represented by B include adenine, guanine, cytosine, thymine, and uracil and modified bases such as 7-deazaguanine, 7-deaza-8-azaguanine, 5-propynylcytosine, 5-propynyluricil, 7-deazaadenine, 7-deaza-8-azaadenine, 7-deaza-6-oxopurine, 6-oxopurine, 3-deazaadenosine, 2-oxo-5-methylpyrimidine, 2-oxo-4-methylthio-5-methylpyrimidine, 2-thiocarbonyl-4-oxo-5-methylpyrimidine, 4-oxo-5-methylpyrimidine, 2-amino-purine, 5-fluorouricil, 2,6-diaminopurine, 8-aminopurine, 4-triazolo-5-methylthymine, 4-triazolo-5-methyluricil and hypoxanthine.

According to a second aspect of the present invention, there is provided a process for the preparation of a compound of Formula (1) as defined above, which comprises oxidising or sulfurising a compound of formula (3):



Formula (3)

wherein R^1, R^2, R^3, R^4, R^5, X^a and X^1 are as defined above.

Compounds of Formula (3) form another aspect of the present invention.

The sulfurization agent employed in the process according to the second aspect of the present invention is any agent able to add sulfur to compounds of Formula (3).

Preferably the sulfurization agent is an organic sulfurization agent.

Examples of organic sulfurization agents include *3H*-benzodithiol-3-one 1,1-dioxide (also called "Beaucage reagent"), dibenzoyl tetrasulfide, phenylacetyl disulfide, N,N,N',N'-tetraethylthiuram disulfide, elemental sulfur, and 3-amino-[1,2,4]dithiazole-5-thione (see U.S. Patent No. 6,096,881, the entire teachings of which are incorporated herein by reference).

Typical reaction conditions for sulfurization of an oligonucleotide using the above agents can be found in Beaucage, *et al.*, *Tetrahedron* (1993), *49*:6123, which is incorporated herein by reference.

Preferred sulfurization reagents are 3-amino-[1,2,4]dithiazole-5-thione and phenylacetyl disulfide.

Sulfurization of an oligonucleotide may be carried out by, for example use of a solution of 3-amino-[1,2,4]dithiazole-5-thione in an organic solvent, such pyridine/acetonitrile (1:9) mixture or pyridine, having a concentration of about 0.05 M to about 0.2 M.

The oxidising agent employed in the process according to the second aspect of the present invention is any agent able to add oxygen to compounds of Formula (3). Examples of oxidising agents include iodine and peroxides, such as t-butylhydroperoxide

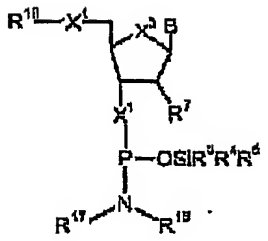
Compounds of formulae (1), (2) and (3) may be prepared by the use of phosphoramidite chemistry, employing silyl phosphoramidites. Accordingly, a third aspect of the present invention comprises compounds of formula (4):

$$R^1\text{-}X^1\text{-}P(NR^{17}R^{18})\text{-}X^a\text{-}SiR^3R^4R^5$$

wherein R^1, R^3, R^4, R^5, X^a and X^1 are as previously defined, and R^{17} and R^{18} are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl. Alternatively, R^{17} and R^{18} taken together with the nitrogen to which they are bound form a heterocyclyl group.

Preferred compounds of the third aspect of the present invention are compounds

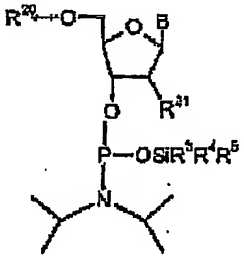
of formula (5):



Formula (5)

wherein R^3, R^4, R^5, R^7, R^{17}, R^{19}, B, X^1 and X^3 are as previously defined, and R^{19} represents an alcohol, thiol or amino protecting group, preferably a protecting group removable under conditions orthogonal to the $OSiR^3R^4R^5$ group. In many embodiments, it is preferred that R^{17} and R^{19} are each alkyl groups, preferably C_{1-4} alkyl groups, and especially isopropyl groups.

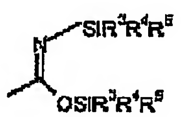
Preferred compounds of formula (5) are compounds of formula (6):



Formula (6)

wherein R^3, R^4, R^5 and B are as previously defined, R^{20} represents a protecting group, preferably a protecting group removable under conditions orthogonal to the group of formula O-$SiR^3R^4R^5$, such as a carbonate protecting group, especially BOC or FMOC, and R^{21} represents H, OMe, $OCH_2CH_2OCH_3$, or OR^{22}, and R^{22} represents a protecting group, known in the art for the protection of the 2'-hydroxy of ribonucleosides, and preferably a silyl, particularly a trialkylsilyl, and especially a tert-butyldimethylsilyl group. In particularly preferred compounds of formula (6), R^3 and R^4 represent methyl groups, and R^5 represents a tert-butyl group. In certain embodiments, especially where a compound of formula (6) is employed to add the final nucleoside of a given oligonucleotide sequence, R^{20} may represent a silyl protecting group, particularly a trialkylsilyl, and especially a tert-butyldimethylsilyl group.

Compounds of formula (4) wherein X^a is O can be prepared by a) reaction between a compound of formula R^1-X^1-H, wherein R^1 and X^1 are as previously defined, and a compound of formula Z-P($NR^{17}R^{18}$)$_2$ wherein R^{17} and R^{18} are as previously defined and Z represents a leaving group, preferably a chlorine atom, to form a compound of formula R^1-X^1-P($NR^{17}R^{18}$)$_2$; b) hydrolysing the compound of formula R^1-X^1-P($NR^{17}R^{18}$)$_2$ to form a compound of formula R^1-X^1-PH(=O)($NR^{17}R^{18}$), the hydrolysis preferably taking place in the presence of a weak acid, such as tetrazole, S-ethyltetrazole, or an imidazole salt; and c) reacting the compound of formula R^1-X^1-PH(=O)($NR^{17}R^{18}$) with a silylating agent of formula Y^1-Si$R^3R^4R^5$ wherein Y^1 is a leaving group to form the compound of formula (4). Examples of leaving groups which can be represented by Y include halogen, especially Cl and Br. Further examples of leaving groups include the residues from bis silylating agents, such as compounds of the formula :



and ($R^3R^4R^5$Si)$_2$NH

wherein R^3, R^4 and R^5 are as previously defined.

Compounds of formula (4) can also be prepared by reaction between a compound of formula R^1-X^1-H, wherein R^1 and X^1 are as previously defined, and a compound of formula $R^3R^4R^5$Si-X^a-P($NR^{17}R^{18}$)$_2$ wherein X^a, R^3, R^4, R^5, R^{17} and R^{18} are as previously defined. The compound of formula $R^3R^4R^5$Si-X^a-P($NR^{17}R^{18}$)$_2$ can be prepared by reaction between a compound of formula Z-P($NR^{17}R^{18}$)$_2$, where Z is as previously defined, and a compound of formula H-X^a-Si$R^3R^4R^5$, preferably in the presence of a base, especially a trialkylamine. Compounds of formula $R^3R^4R^5$Si-O-P($NR^{17}R^{18}$)$_2$ may also be prepared by hydrolysis of a compound of formula Z-P($NR^{17}R^{18}$)$_2$, to form a compound of formula H-O-P($NR^{17}R^{18}$)$_2$, which is then reacted with a compound of formula Y^1-Si$R^3R^4R^5$ wherein Y^1 is as described above.

According to a fourth aspect of the present invention, there is provided a process for the preparation of a compound of formula (1) which comprises a) coupling a compound of formula (4) as defined above with a nucleoside or oligonucleotide comprising a free hydroxy or thiol group, of formula R^2-X^1-H wherein R^2 and X^1 are as previously defined, and preferably a nucleoside or oligonucleotide comprising a free 5'-hydroxy group, in the presence of an activator, and b) oxidising or sulfurising the product of step a). In one embodiment, the process of the fourth aspect of the present invention comprises the coupling of a compound of formula (4) as defined above to add the final nucleoside in an oligonucleotide, the remaining nucleosides of which having been added using phosphoramidites comprising conventional phosphorus protecting groups, such as betacyanoethyloxy phosphoramidites.

Preferably the nucleoside or oligonucleotide comprising the free hydroxyl or thiol group is attached to a solid support, most preferably via a cleavable linker, preferably a trityl or succinyl linker. It is particularly preferred that the attachment to the solid support is via the 3'-position of a ribose or deoxyribose moiety.

A preferred embodiment of the present invention comprises a sequence of processes of the fourth aspect wherein a series of compounds of formula (4) are sequentially coupled to a free hydroxy group to form a nascent oligonucleotide, a protecting group, most preferably a 5'-protecting group, is removed from the nascent oligonucleotide to form a free hydroxy group, which is then coupled with another compound of Formula (4) in the presence of an activator. The cycle can be repeated as often as desired until the desired oligonucelotide sequence has been assembled.

The compound of formula (4) is advantageously employed as a solution in an inert solvent. Examples of such solvents suitable for use in phosphoramidite chemistry are well known in the art, and include in particular acetonitrile, dichloromethane, THF and pyridine.

Activators which can be employed in the process of the present invention are well known in the filed of oligonucleotide synthesis. Examples include tetrazole; S-ethyl tetrazole; pyridinium salts, imidazolinium salts and benzimidazolinium salts as disclosed in PCT application WO 99/62922 (incorporated herein by reference) and salt complexes formed between saccharin and organic amines, especially N-methylimidazole, pyridine and 3-methylpyridine.

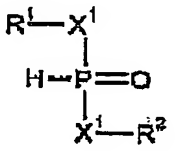
According to a fifth aspect of the present invention, there is provided a process for the preparation of a deprotected oligonucleotide which comprises a) assembling a compound by a process according to the fourth aspect of the present invention, and removing the $SiR^3R^4R^5$ groups. The $SiR^3R^4R^5$ groups can be removed by methods known in the art for the removal of organosilyl protecting groups, for example by treatment with a source of fluoride, such as ammonium fluoride, under basic, nucleophilic conditions. The $SiR^3R^4R^5$ groups can be removed either before or after other protecting groups are removed. It will be recognised that this, together with the nature of the other protecting groups, may influence the choice of conditions employed. For example, the $SiR^3R^4R^5$ groups may be removed by treatment with acetic acid, which treatment will also remove trityl-type protecting groups. When the oligonucleotide has been prepared whilst supported on a solid support, the $SiR^3R^4R^5$ groups are commonly removed after cleavage of the oligonucleotide from the support.

A sixth aspect of the present invention provides a process for the synthesis of an oligonucleotide comprising at least one internucleotide phosphorus atom protected with a group of formula $-X^1SiR^3R^4R^5$, wherein X^1 represents O or S, and R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more which comprises reacting a silylating agent of formula Y^1-$SiR^3R^4R^5$ as described above with an oligonucleotide H-

phosphonate diester.

Particularly preferred trihydrocarbylsilyl donors are *tert*-butyldimethylsilyl chloride, bis(*tert*-butyldimethylsilyl) acetamide and bis(tert-butyldimethyl)disilazane.

Preferred oligonucleotide H-phosphonate diesters are compounds of formula (7):



Formula (7)

wherein R^1, R^2 and X^1 are as previously defined.

Oligonucleotide H-phosphonate diesters can be prepared by methods well known in the art, for example by reaction between a nucleoside or oligonucleotide H-phosphonate monoester, and a nucleoside or oligonucleotide comprising a free hydroxylor thiol group.

Solvents which may be employed in the processes of the resent invention include: haloalkanes, particularly dichloromethane; esters, particularly alkyl esters such as ethyl acetate, and methyl or ethyl propionate; nitriles, such as acetonitrile; amides, such as dimethylformamide and N-methylpyrollidinone; and basic, nucleophilic solvents such as pyridine. Preferred solvents are pyridine, dichloromethane, dimethylformamide, N-methylpyrollidinone and mixtures thereof. A particularly preferred solvent is pyridine. Organic solvents employed in the process of the present invention are preferably substantially anhydrous.

Supports for the solid phase synthesis of oligonucleotides are well known in the art. Examples include silica, controlled pore glass, polystyrene, copolymers comprising polystyrene such as polystyrene-poly(ethylene glycol) copolymers and polymers such as polyvinylacetate. Additionally, poly(acrylamide) supports, especially microporous or soft gel supports, such as those more commonly employed for the solid phase synthesis of peptides may be employed if desired. Preferred poly(acrylamide) supports are amine-functionalised supports, especially those derived from supports prepared by copolymerisation of acryloyl-sarcosine methyl ester, N,N-dimethylacrylamide and bis-acryloylethylenediamine, such as the commercially available (Polymer Laboratories) support sold under the catalogue name PL-DMA. The procedure for preparation of the supports has been described by Atherton, E.; Sheppard, R. C.; in *Solid Phase Synthesis: A Practical Approach*, Publ., IRL Press at Oxford University Press (1984) which is incorporated herein by reference. The functional group on such supports is a methyl ester and this is initially converted to a primary amine functionality by reaction with an alkyl

diamine, such as ethylene diamine.

The processes for the synthesis of a trihydrocarbyl silyl phosphorothioate triester in the solid state may be carried out by stirring a slurry of the substrate bonded to the solid and comprising silyl phosphite linkages in a solution of sulfurization agent. Alternatively, the solid support can be packed into a column, and solutions of the sulfurization agent can be passed through the column.

The processes according to the present invention are preferably employed to produce oligonucleotides comprising at least one internucleotide phosphorus atom protected with a group of formula $-X^1SiR^5R^4R^6$ as defined above, which comprise 3 or more bases. Preferably the oligonucleotide comprises 5 to 75, more preferably from 8 to 50 and particularly from 10 to 30 internucleoside linkages. Commonly, the processes of the present invention are employed to prepare compounds wherein at least 50% of the internucleoside linkages are phosphorothioated, preferably at least 75%, and most preferably 90 to 100% of the internucleoside linkages phosphorothioated.

When the processes according to the present invention are used to produce oligonucleotides then the conditions used are any of those known in the art.

On completion of the assembly of the desired product, the product may be cleaved from the solid support, using cleavage methods appropriate for the linker, preferably following deprotection of the product.

The trihydrocarbyl silyl phosphorothioate product of the process can be purified using one or more standard techniques known in the art, such as, ion-exchange chromatography, reverse phase chromatography, precipitation from an appropriate solvent and ultra-filtration.

Many of the compounds used herein may exist in the form of a salt. These salts are included within the scope of the present inventions.

The compounds described herein may exist in tautomeric forms other than those shown in this specification. These tautomers are also included within the scope of the present inventions.

The invention will now be illustrated without limitation by the following examples.

Liquid Chromatography Analysis

In the examples analysis by liquid chromatography used the following protocol:

All samples were prepared in acetonitrile;
The chromatography media was Genesis C18, 120A, 4μ;
The dimensions of the column were 25 x 0.46 cm;
The flow rate was1.0 ml / minutes;
The detector was set at 270 nm;

The run time was 30 minutes;
The elution system used the following solvents:
0 minutes = 80% 0.1% aqueous ammonium acetate buffer: 20% acetonitrile
20 minutes = 100% acetonitrile
22 minutes = 100% acetonitrile
30 minutes = 80% 0.1% aqueous ammonium acetate buffer: 20% acetonitrile.

In the examples the following abbreviations are used:

BMTBSA	*N,O*-Bis(*tert*-butyldimethylsilyl)acetamide
BSA	*N,O*-Bis(trimethylsilyl)acetamide
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMT	4,4'-Dimethoxytrityl
PADS	Diphenyldithiocarbamate
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TEAP	Triethylamine phosphate
THF	Tetrahydrofuran
TMS	Trimethyl silyl

Example 1

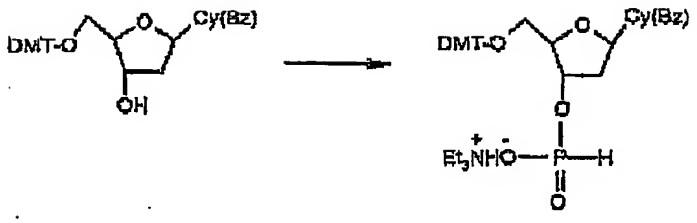
Stage 1

Preparation of 3M aqueous triethylamine phosphate (TEAP)

Triethylamine (410 ml) and water (400 ml) were charged to a beaker and cooled to 0 - 5°C. Phosphoric acid (180 g) was added slowly to the stirred mixture until the pH was in the range of pH 7 to 7.5 was reached. The solution was then transferred to a 1L volumetric flask and diluted to 1L with water. Prior to use TEAP was diluted with water as required.

Stage 2

Preparation of N^4-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-3'-(hydrogen phosphate)cytidine triethylammonium salt (DMT-Bz-C-H-Phos)

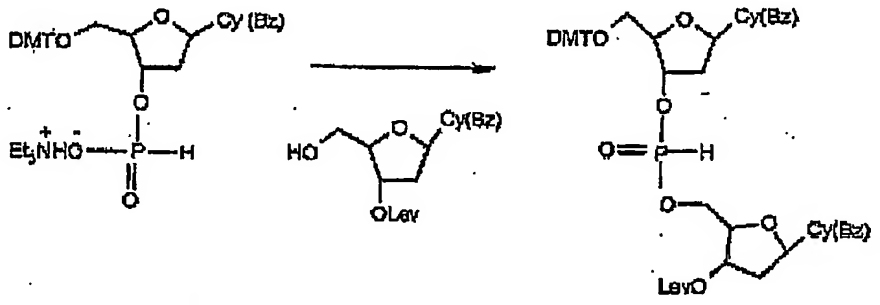


DMT-Bz-C-OH DMT-Bz-C-H-Phos

THF (416 ml) and 1H,1,2,4-triazole (16.1 g) were charged to a 1L round-bottomed flask fitted with a thermometer, condenser, nitrogen inlet and overhead stirrer. The solution was cooled, with stirring, to -10°C. Triethylamine (32.2 g, 44.35 ml) was added in one portion followed by the dropwise addition of PCl_3 (6.7 ml) while maintaining the reaction temperature between -15 to -10°C. The reaction mixture was further stirred for 0.5 h at -15 to -10°C. N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (DMT-Bz-C-OH) (12.4 g, from Transgenomic Bioconsumables Ltd) in THF (347 ml) was added to the reaction mixture over a 1 h period and the mixture was then stirred at -10°C for a further period of 1 h. The reaction mixture was then added to a stirred mixture of triethylamine : H_2O, (1:1, 200 ml) at -10°C over a period of 15 minutes and allowed to warm to room temperature before being transferred to a separating funnel. The bottom layer was discarded while the top layer was concentrated *in vacuo*. DCM (580 ml) was added to the residue and the resulting solution was washed with TEAP (0.5 M, 2 x 75 ml). The reaction mixture was concentrated *in vacuo* to yield 14.75 g of product (94% yield).

Stage 3

Synthesis of N^4-benzoyl-5'-O-(4,4'-dimethoxytrityl)cytidin-3'-yl-N^4-benzoyl-2'-deoxy-3'-(4-oxopentanoate)-cytidin-5'-yl H-phosphonate (C-C dimer)



DMT-Bz-C-H-Phos HO-Bz-C-OLev C-C dimer

Prior to use all glassware was dried in an oven and cooled in a desiccator. N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-3'-(hydrogen phosphate)cytidine triethylammonium salt (DMT-Bz-C-H-Phos) (1.1 g, prepared as described in Stage 2) and

N^4-benzoyl-2'-deoxy-3'-(4-oxopentanoate)cytidine (HO-Bz-C-OLev) (0.5 g, from Transgenomic Bioconsumables Ltd) were dried from an azeotropic mixture with CH_3CN (2 x 25 ml) and toluene (25 ml). The residue was transferred to a 50 ml round-bottomed flask fitted with a nitrogen inlet and dry DMF (10 ml) and dry pyridine (0.56 ml) were added. The mixture was cooled to 0°C and diphenyl chlorophosphate (0.59 ml in dry DCM (3 ml)) was added dropwise over 2 minutes. The reaction was held at 0°C for 15 minutes before being quenched by the addition of pH 7 phosphate buffer (5 ml, supplied by Fisher). Saturated aqueous $NaHCO_3$ (40 ml) was then added to the mixture followed by DCM (40 ml). The lower organic layer was separated and washed with TEAP (0.5 M, 30 ml) and then dried over Na_2SO_4. The title compound (C-C dimer) was stored as a dried DCM solution over Na_2SO_4 in a nitrogen flushed flask at 4°C to minimise decomposition. Coupling of DMT-Bz-C-H-Phos and HO-Bz-C-OLev to provide the C-C dimer was quantitative by liquid chromatography. However, the C-C dimer, as produced also contained as impurities unreacted pyridine, DMF and $(PhO)_2P(O)(OH)$. Therefore in subsequent experiments the calculated mass of C-C dimer was proportionally increased to compensate for the additional components present within the crude material.

Prior to use the C-C dimer mixture was filtered to remove Na_2SO_4 and concentrated *in vacuo*.

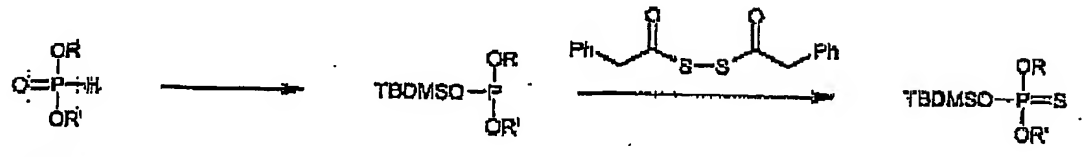
Stage 4

Preparation of *N,O*-bis(*tert*-butyldimethylsilyl)acetamide (BMTBSA)

Prior to use all glassware was dried in an oven and cooled in a desiccator. Acetamide (7.13 g) was charged to a 1L round-bottomed flask fitted with a thermometer, nitrogen inlet and overhead stirrer. Dry triethylamine (340 ml, pre-dried over CaH_2) was added and the solution was cooled to 0°C. TBDMSCl (47.37 g) was then added with vigorous stirring. The reaction mixture was vigorously stirred for 22 h and then filtered under nitrogen using dried glassware before being concentrated *in vacuo*. The resultant crude product mixture was distilled using a Kugelrohr apparatus under 0.6 - 0.8 mm Hg pressure and at a temperature of from 85 to 100°C. The distilled material solidified to a white solid (14.85 g) which was postulated to be a 2:1 mixture of the di- and mono-silylated acetamide. This was determined from ^1H NMR analysis where the major component was identified as BMTBSA giving signals in agreement with those reported in the literature (*J. Org. Chem*, **1982**, *47*, 3336-3339). The minor component contained one TBDMS functional group with ^1H NMR signals consistent with those expected for the mono-silylated acetamide. The mono-silylated acetamide was assumed to be of similar activity to BMTBSA, therefore in subsequent experiments the mass of BMTBSA used was calculated based on the assumption that the crude BMTBSA material was 100% pure.

Stage 5

Reaction of the C-C dimer with BMTBSA and PADS



C-C dimer

1

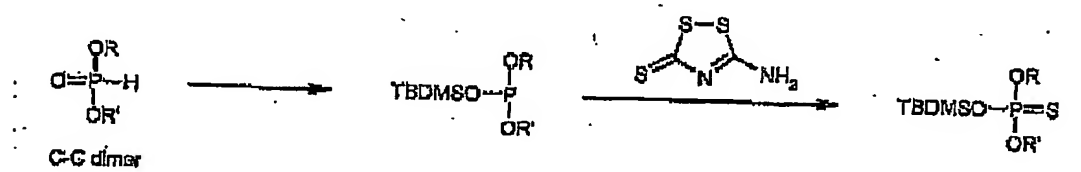
Prior to use all glassware was dried in an oven and cooled in a desiccator. BMTBSA was warmed to melt the solid and was then measured by volume in an air tight syringe which had been heated in the oven immediately prior to use to prevent solidification of the solid (the density of BMTBSA was taken as d=0.869 (*J. Org. Chem*, 1982, *47*, 3336-3339)).

C-C dimer (1.007 g, prepared as described in Example 1, Stage 1 and Stage 2) was charged to a 25 ml round-bottomed flask fitted with nitrogen inlet and dissolved in dry DCM (5 ml). BMTBSA (0.90 ml, 5 equiv, prepared in Example 1, Stage 3) was added to the flask. The reaction mixture was stirred for 5 minutes. PADS (327 mg, 2 equivalents, from Hasegawa Co., Ltd) was then added and the mixture was stirred for a further 5 minutes. During this time the reaction mixture changed from a yellow to a deep purple solution. The reaction mixture was poured onto water (100 ml) and the organic layer was separated. The aqueous layer was further extracted with DCM (3 x 50ml). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (2 x 50ml) and brine (2 x 50ml) and dried over Na_2SO_4. Filtration and concentration *in vacuo* gave 1.82 g of a purple liquid which solidified on standing.

The crude product was analysed by liquid chromatography where the product (1) retention time was 11.1 minutes (17%).

Example 2

Reaction of the C-C dimer with BMTBSA and 3-amino-1,2,4-dithiazole-5-thione



C-C dimer

1

Prior to use all glassware was dried in an oven and cooled in a desiccator. C-C dimer (1.007 g, prepared as described in Example 1, Stage 1 and Stage 2) was charged

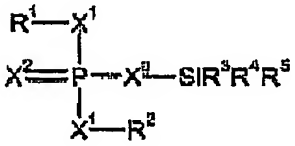
to a 25 ml round-bottomed flask fitted with a nitrogen inlet and dissolved in dry DCM (5 ml). BMTBSA (0.90 ml, 5 equiv, prepared in Example 1, Stage 3) was then added to the flask and the reaction mixture was stirred for 5 minutes. 3-Amino-1,2,4-dithiazole-5-thione (162 mg, 2 equivalents from Lancaster) was then added and stirring was continued for a further 5 minutes. The reaction mixture was poured onto water (100 ml) and the organic layer was separated. The aqueous layer was further extracted with DCM (3 x 50ml). Organic layers were combined and washed with saturated aqueous $NaHCO_3$ (2 x 50ml) and brine (2 x 50ml) and dried over Na_2SO_4. Filtration and concentration *in vacuo* gave 1.10 g of a pale yellow solid.

The crude product was analysed by liquid chromatography and the main product was identified as compound (1) (68% yield) which had a retention time of 10.9 minutes in the liquid chromatography system described above.

CLAIMS

1. An oligonucleotide comprising at least one internucleotide phosphorus atom protected with a group of formula $-X^aSiR^3R^4R^5$ wherein X^a represent O or S, and R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more.

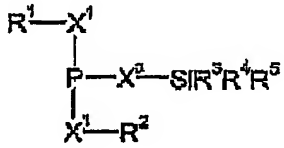
2. A compound according to claim 1, having the Formula(1):



Formula (1)

wherein:
R^1 and R^2 independently are nucleoside or oligonucleotide moieties;
R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more;
X^a represents O or S, preferably O;
each X^1 independently is O, S or NR^n, where R^n represents H or C_{1-4} alkyl, preferably each X^1 being O; and
X^2 is O or S, preferably S.

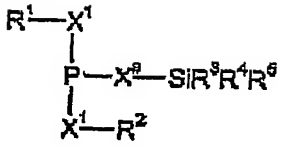
3. A process for the preparation of a compound of Formula (1) as defined in claim 2, which comprises oxidising or sulfurising a compound of formula (3):



Formula (3)

wherein R^1, R^2, R^3, R^4, R^5, X^a and X^1 are as defined in claim 2.

4. A compound of formula (3):



Formula (3)

wherein R^1, R^2, R^3, R^4, R^5, X^a and X^1 are as defined in claim 2.

5. A compound of formula (4):

$$R^1\text{-}X^1\text{-}P(NR^{17}R^{18})\text{-}X^a\text{-}SiR^3R^4R^5$$

wherein R^1, R^3, R^4, R^5, X^a and X^1 are as defined in claim 2, and R^{17} and R^{18} are each, independently, a substituted or unsubstituted aliphatic group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl or R^{17} and R^{18} taken together with the nitrogen to which they are bound form a heterocyclyl group.

6. A process for the preparation of a compound of formula (1) as defined in claim 1 which comprises:
a) coupling a compound of formula (4) as defined in claim 5, with a compound of formula $R^2\text{-}X^1\text{-}H$ wherein R^2 and X^1 are as defined in claim 1, in the presence of an activator; and
b) oxidising or sulfurising the product of step a).

7. A process for the preparation of a compound of formula (3) as defined in claim 4 which comprises coupling a compound of formula (4) as defined in claim 5, with a compound of formula $R^2\text{-}X^1\text{-}H$ wherein R^2 and X^1 are as defined in claim 1, in the presence of an activator.

8. A process for the preparation of a compound of formula (4) as defined in claim 5, which comprises reacting a compound of formula $R^1\text{-}X^1\text{-}H$, wherein R^1 and X^1 are as defined in claim 1 with a compound of formula $R^3R^4R^5Si\text{-}X^a\text{-}P(NR^{17}R^{18})_2$ wherein X^a, R^3, R^4, R^5, R^{17} and R^{18} are as defined in claim 5.

9. A process for the preparation of a compound of formula (4) wherein X^a is O which comprises a) reacting a compound of formula $R^1\text{-}X^1\text{-}H$, wherein R^1 and X^1 are as defined in claim 1 and a compound of formula $Z\text{-}P(NR^{17}R^{18})_2$ wherein R^{17} and R^{18} are as defined in claim 5 and Z represents a leaving group, preferably a chlorine atom, to form a compound of formula $R^1\text{-}X^1\text{-}P(NR^{17}R^{18})_2$; b) hydrolysing the compound of formula $R^1\text{-}X^1\text{-}$

$P(NR^{17}R^{18})_2$ to form a compound of formula R^1-X^1-$PH(=O)(NR^{17}R^{18})$, the hydrolysis preferably taking place in the presence of a weak acid, such as tetrazole, S-ethyltetrazole, or an imidazole salt; and c) reacting the compound of formula R^1-X^1-$PH(=O)(NR^{17}R^{18})$ with a silylating agent of formula Y^1-$SiR^3R^4R^5$ wherein Y^1 is a leaving group, to form the compound of formula (4).

10. A process for the preparation of a compound of formula $R^3R^4R^5Si$-X^a-$P(NR^{17}R^{18})_2$ which comprises reaction of a compound of formula Z-$P(NR^{17}R^{18})_2$ as defined in claim 9, with a compound of formula H-X^a-$SiR^3R^4R^5$, wherein X^a, R^3, R^4, and R^5 are as defined in claim 1, preferably in the presence of a base.

11. A process for the preparation of a compound of formula $R^3R^4R^5Si$-O-$P(NR^{17}R^{18})_2$ wherein R^3, R^4, and R^5 are as defined in claim 1, and R^{17} and R^{18} are as defined in claim 5 which comprises:

a) hydrolysis of a compound of formula Z-$P(NR^{17}R^{18})_2$ wherein Z is as defined in claim 9 to form a compound of formula H-O-$P(NR^{17}R^{18})_2$; and

b) reaction of the product of step a) with a compound of formula Y^1-$SiR^3R^4R^5$ wherein Y^1 is a leaving group.

12. A process for the synthesis of an oligonucleotide comprising at least one internucleotide phosphorus atom protected with a group of formula -$X^1SiR^3R^4R^5$, wherein X^1 represents O or S, and R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more, which comprises reacting a silylating agent of formula Y^1-$SiR^3R^4R^5$ wherein Y^1 is a leaving group with an oligonucleotide H-phosphonate diester.

ABSTRACT
PROCESS AND COMPOUNDS

Oligonucleotide comprising at least one internucleotide phosphorus atom protected with a group of formula $-X^aSiR^3R^4R^5$ are provided. X^a represent O or S, and R^3, R^4 and R^5 each independently are optionally substituted hydrocarbyl groups, selected such that that total number of carbon atoms in R^3 plus R^4 plus R^5 is 4 or more. Process for the preparation of these oligonucleotides, intermediate compounds useful therein, and process for the preparation of the intermediate compounds are also provided.